Feeding inhibition by urocortin in the rat hypothalamic paraventricular nucleus

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Wang, Chuanfeng, Mary A. Mullett, Michael J. Glass, Charles J. Billington, Allen S. Levine, and Catherine M. Kotz. Feeding inhibition by urocortin in the rat hypothalamic paraventricular nucleus. Am J Physiol Regulatory Integrative Comp Physiol 280: R473–R480, 2001.—Ventricular administration of urocortin (UCN) inhibits feeding, but specific site(s) of UCN action are unknown. In the current studies we examined the effect of UCN in the hypothalamic paraventricular nucleus (PVN) on feeding. We tested UCN administered into the PVN in several paradigms: deprivation-induced, nocturnal, and neuropeptide Y (NPY)-induced feeding. We compared the effect of equimolar doses of UCN and corticotrophin releasing hormone (CRH) on NPY-induced and nocturnal feeding, determined whether UCN in the PVN produced a conditioned taste aversion (CTA) and induced changes in c-Fos immunoreactivity (c-Fos-ir) after UCN and NPY administration in the PVN. UCN in the PVN significantly decreased NPY and nocturnal and deprivation-induced feeding at doses of 1, 10, and 100 pmol, respectively. UCN anorectic effects lasted longer than those attributed to CRH. Ten and thirty picomoles UCN did not induce a CTA, whereas 100 pmol UCN produced a CTA. UCN (100 pmol) in the PVN neither increased c-Fos-ir in any brain region assayed nor altered c-Fos-ir patterns resulting from NPY administration. These data suggest the hypothalamic PVN as a site of UCN action.

energy balance; neuropeptide Y; feeding behavior; brain sites

UCN (UCN) is a recently identified peptide that has 45% sequence identity with corticotrophin releasing hormone (CRH) and binds to all three CRH receptors (CRH-R1, CRH-R2α, and CRH-R2β) with 6×, 20×, and 40× higher affinity than CRH, respectively (13). CRH receptors are widely distributed throughout the central nervous system (CNS; Ref. 1), with moderate levels of CRH-R2 receptors in the hypothalamic paraventricular nucleus (PVN) (2). Intracerebroventricular injection of UCN potently and dose dependently decreases feeding in food-deprived and free-feeding rats (12) and appears to be more potent than CRH in decreasing feeding. The ED50 for UCN is 0.27 μg (intracerebroventricularly), whereas the ED50 for CRH is 6.82 μg (intracerebroventricularly) (12). Meal patterning of rats receiving central UCN indicates that low doses of UCN decrease meal size without decreasing the frequency of meals, a behavioral pattern similar to that after low-dose administration of d-fenfluramine (12). Food deprivation results in decreased UCN fibers and varicosities in the PVN (3), which is consistent with the anorectic properties of UCN, and suggests that UCN may be part of a feeding regulatory network.

Although previous studies indicate that UCN decreases feeding (12), the ventricular injection limits interpretation of the results, because the neural origin of UCN stimulus is unknown. The purpose of the current studies was to determine the effect of UCN administered directly into the PVN on feeding. On the basis of the satiety effect of UCN administered ventricularrly and the central role of the PVN in feeding and energy metabolism, we hypothesized that UCN in the PVN is anorectic. Within the PVN, UCN immunoreactivity has been demonstrated in perikarya and fibers (3, 5), and the predominant CRH receptor subtype in the PVN is CRH-R2 (2), which is the subtype to which UCN binds with the highest affinity (13). These factors provide a neuroanatomic basis for UCN action in the PVN (3, 5). To determine whether UCN in the PVN has a role in feeding, we tested the effect of UCN in the PVN in several feeding paradigms: deprivation-induced, nocturnal, and NPY-induced feeding. Because of the close association between UCN and CRH, we performed two studies to compare the effectiveness of equimolar doses of UCN and CRH in the PVN on feeding inhibition. Because there is the possibility that anorectic agents reduce feeding through an alternate mechanism, such as making an animal feel ill, we also assessed whether UCN administration in the PVN results in a conditioned taste aversion (CTA). Finally, we tested whether UCN induced changes in c-Fos immunoreactivity (c-Fos-ir, marker of early gene activation) in several feeding-regulatory brain sites after UCN and NPY administration in the PVN, to deter-

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mine whether UCN influences neuronal signaling in these brain regions.

MATERIALS AND METHODS

Animals

In all experiments, male Sprague-Dawley rats (Harlan, Madison, WI, or Charles River, Portage, MI) weighing 250–350 g were individually housed in conventional hanging cages with a 12:12-h light-dark photoperiod (lights on at 0700) in a temperature-controlled room (21–22°C). Purina-verified lab chow and water were allowed ad libitum, except where noted.

Cannulation

Rats were anesthetized with Nembutal (40 mg/kg) and fitted with 26-gauge stainless steel guide cannulas (Plastics One, Roanoke, VA) placed unilaterally 1 mm above the PVN target for all experiments to avoid damage to the PVN by the guide cannula. Stereotaxic coordinates were determined from the rat brain atlas by Paxinos and Watson (8) and were as follows: PVN 0.5 mm lateral, 1.9 mm posterior to bregma, and 7.3 mm below the skull surface. For all cannulations, the incisor bar was set at 3.3 mm below the ear bars. At least 7 days elapsed after surgery before experimental trials.

Injections

Injections into the PVN were given in a 0.5-μl volume over 30 s by the use of a 33-gauge internal cannula (Plastics One, Roanoke, VA). In studies where more than one injection was given, there was at least a 60-s delay between injections. The injector extended 1 mm beyond the end of the guide cannula.

Verification of Cannula Placement

After experiments 1–2 and 4, brains were dissected out and stored in a 10% formaldehyde solution for later placement verification by histologic examination. Data from animals with incorrectly placed cannulas were excluded from the final analysis. The number of rats listed in the specific experimental protocols represent the number of rats in the final analysis (all cannulas correctly placed). In experiments 3A and 3B, which involved injection of NPY into the PVN, we used the feeding response to NPY as a biomarker for correct PVN cannula placement. This is based on our experience with comparisons of PVN cannula placement and feeding response to PVN-injected NPY. Additionally, if an animal does not respond to NPY, then a treatment effect (i.e., UCN or CRH) on NPY-induced feeding cannot be assessed. Thus if an animal consumed <1.8 g after PVN NPY injection, then all data from this animal were removed from the data analysis. In experiment 5, placement was verified during tissue analysis. Although injections were performed to minimally perturb the PVN tissue, stained PVN tissue slices from animals receiving several injections over time were examined for possible tissue damage under a light microscope. From these preparations, no apparent damage was visible.

Drugs

UCN and CRH were purchased from Phoenix Pharmaceuticals (Mountain View, CA). Porcine NPY was purchased from Peninsula Laboratories (Belmont, CA). All drugs were dissolved in artificial cerebrospinal fluid (aCSF) just before use.

Food Intake Measurements

Food was allowed ad libitum until the start of each experimental trial. Just before injection, food was removed, and, immediately after injection, preweighed pellets of chow were placed inside the rat cage. At selected time points, pellets and spillage were weighed and subtracted from the initial weight to quantify the amount of food eaten.

Two Bottle Preference Test

The two bottle preference test developed by Richter and colleagues (10, 11) was used to determine whether UCN is aversive when administered into the PVN. The basis for this is as follows: when rats are exposed to water and saccharin solutions they show a preference for saccharin. When these animals are then injected with a drug that has aversive properties, saccharin drinking is associated with the aversive stimuli, and, on subsequent testing, drinking of saccharin is reduced. Thus reduced consumption of the drug-paired flavor indicates that the previously preferred flavor has now become aversive as a result of drug administration.

Immunohistochemical Procedures

Animals were anesthetized with pentobarbital sodium (60 mg/kg ip), killed by cardiac puncture, and rapidly perfused through the aorta with 80–100 ml of 0.9% saline. Animals were then slowly perfused with 500 ml of Lana’s Fix (4% paraformaldehyde-14% picric acid-PBS). Brains were removed, stored in 10% sucrose-Sorenson’s overnight at 4°C, and blocked, and 40-μm sections were cut through the areas of interest. Immediately after being sliced, brain sections were placed in cryoprotectant (polyvinylpyrrolidone-40, 30% ethylene glycol-10% sucrose in phosphate buffer) and stored at −20°C until use. Storage of tissue in this manner has been shown to preserve morphology for up to 90 days (14).

Tissue sections were removed from cryoprotectant and rinsed in PBS 4 × 5 min, incubated in blocking serum (2% normal goat serum, 0.1% BSA, 0.2% Triton X-100 in PBS) for 20 min, and rinsed again in PBS 3 × 5 min. Sections were then slowly perfused with 500 ml of Lana’s Fix (4% paraformaldehyde-14% picric acid-PBS) for 20 min, and rinsed again in PBS 3 × 5 min. Sections were then incubated in rabbit anti-c-Fos IgG (Oncogene Science, Uniondale, NY) at a 1:50,000 dilution at 4°C for 48 h. Primary antibody was removed, and sections were washed 6–8 × 5 min in PBS and incubated in biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) for 1 h at room temperature. Secondary antibody was then removed, and sections were rinsed 4 × 5 min in PBS and incubated in Vectastain Elite ABC reagent (Vector Laboratories) for 1 h at room temperature. The avidin-biotin complex reagent was removed, and sections were rinsed 2 × 5 min in PBS and 3 × 5 min in sodium acetate buffer. Sections were incubated with 3,3-diaminobenzidine-nickel sulfate for 2.5 min, after which the reaction was stopped with distilled water, and sections were rinsed more than three times with distilled water.

Stained and mounted sections were viewed via light microscopy with an Optronics (Goleta, CA) TEC-470 cooled charge-coupled device camera mounted on a Leitz Orthoplan 2 microscope. For each site, three slides per animal were selected, and the images were fed into a Power Macintosh G3 computer and analyzed using National Institutes of Health Image 1.51 software. This allows quantification of Fos-ir-positive nuclei within a measured area. The output, which the software gives, is the number of c-Fos-ir-positive nuclei per 0.1 mm². Data from three slices per animal were averaged and represented the value for that animal.
Specific Experimental Protocols

For all repeated-measurement feeding studies (experiments 1, 2, and 3), the order of treatments was counterbalanced, such that each treatment was given to a subset of rats on each day so that injection order and potential day effects would not confound results. Each rat received each treatment once with at least 72 h between each session of treatments to allow for clearance of drug from the CNS. In the nocturnal study, UCN and CRH effects were gone after 40 h, so a 72-h gap between treatments was deemed sufficient to avoid carryover effects.

Experiment 1: the effect of PVN UCN administration on deprivation-induced feeding. Twelve PVN-cannulated rats were food-deprived overnight (18 h). At 0900, rats were microinjected with either aCSF or 10, 30, or 100 pmol UCN. Food intake was measured at 1, 2, 4, and 24 h postinjection.

Experiment 2A: the effect of equimolar doses of UCN and CRH administered in the PVN on nocturnal feeding. At 1830, 30 min before the 12-h time period of lights-off, 14 PVN-cannulated rats were microinjected with either aCSF, 10 pmol UCN, or 10 pmol CRH. Food intake was measured at 1, 2, 4, 16, and 40 h postinjection.

Experiment 2B: dose effect of UCN administered in the PVN on nocturnal feeding. At 1830, 30 min before the 12-h time period of lights-off, eight PVN-cannulated rats were microinjected with either aCSF or 3, 10, 30, or 100 pmol UCN. Food intake was measured 1 h postinjection.

Experiment 3A: the effect of PVN UCN or CRH administration on PVN NPY-induced feeding: dose response. Between 0930 and 1130, nine PVN-cannulated rats were microinjected with either aCSF or 10, 20, or 100 pmol CRH or UCN into the PVN. After 20 min, animals were injected with either 117 pmol NPY or aCSF and allowed free access to food. Food intake was measured (in hoppers) at 1, 2, and 4 h after NPY injection.

Experiment 3B: the effect of PVN UCN administration on PVN NPY-induced feeding: dose response. Between 0930 and 1130, nine PVN-cannulated rats were microinjected with either aCSF or 1, 3, 10, or 30 pmol UCN into the PVN. After 20 min, animals were injected with 117 pmol NPY or aCSF and allowed free access to food. Food intake was measured (in hoppers) at 1, 2, 4, and 24 h after NPY injection.

Experiment 4: the effect of PVN UCN administration on preference for saccharin solution. Rats matched for body weight were assigned to one of four groups: 1) 0 pmol UCN (aCSF = vehicle), 2) 1 pmol UCN, 3) 30 pmol UCN, and 4) 100 pmol UCN, (n = 6 per treatment group). All groups were subject to water restriction. On the paired-training day, rats were provided with 5 ml of 0.1% saccharin to drink. After consumption of the solution, rats were then injected (UCN in 0.5-μl volume administered into the PVN) with their respective training stimulus (i.e., vehicle or UCN), thus experience with the taste stimulus and drug occurred at approximately the same time. On the following day (unpaired-training day), rats were microinjected with vehicle or UCN, where the UCN-saccharin paired rats received vehicle and the aCSF-saccharin rats received UCN, doses of which were chosen at random such that each dose was equally represented; however, on this day, rats were not exposed to the training solution. In this way, both paired and unpaired rats had similar exposure to drugs and saccharin, but only the paired groups received a drug immediately after taste exposure. These two training sessions were repeated in the same order on the subsequent 2 days, and thus paired groups received two sessions where taste was immediately followed by drug. These conditions allow for better conditioning and serve as a more sensitive index of aversion, which is a more desirable situation when dealing with a novel anorectic agent. Three days after the last session, rats were provided with a choice between 0.1% saccharin and water. Consumption was measured over 24 h. A saccharin preference ratio was then calculated as the amount of saccharin consumed divided by total consumption of both saccharin and water. During testing, the water bottle of one rat in the 30-pmol group developed a leak, and this rat’s data were not included in the analysis. To contrast the effects of UCN on CTA with an established aversive stimulus, two separate groups of rats were exposed to vehicle or LiCl (125 mg/kg ip, n = 6 per treatment group). The protocol was similar to that used for the UCN-treated groups, except that these rats received only one paired session, given the ability of LiCl to rapidly stimulate CTA.

Experiment 5: the effect of PVN UCN on c-Fos-ir in several brain regions. Twenty PVN-cannulated rats were injected with either aCSF or 100 pmol UCN. Two hours after the second injection, rats were killed and their brains were processed for c-Fos immunohistochemistry as described in Immunohistochemical Procedures. These regions included the cortical nucleus of the amygdala (ACo), the hypothalamic arcuate nucleus (Arc), the bed nucleus of the stria terminalis (BNST), the central nucleus of the amygdala (CeA), the dorsomedial hypothalamus (DMH), the lateral hypothalamus (LH), the intermediate portion of the lateral septal nucleus (LSi), the PVN, the medial supramammillary nucleus (SUMM), and the ventromedial hypothalamus (VMH).

Statistical Analysis

Experiments 1–3. The data were analyzed by repeated-measures ANOVA; thus each rat served as its own control. When main effects were observed, post hoc analysis was performed using multiple-comparison contrasts.

Experiments 4–6. Data were analyzed by a one-factor ANOVA followed by Fisher’s least-significant difference t-test to compare means.

RESULTS

Experiment 1: The Effect of PVN UCN Administration on Deprivation-Induced Feeding

There was a main effect of UCN on deprivation-induced feeding at 1 and 2 h after injection (0–1 h: F_{3,33} = 2.942, P = 0.0473; 0–2 h: F_{3,33} = 5.328, P = 0.0042; Fig. 1). Post hoc analysis indicated that only the 100-pmol dose of UCN significantly decreased deprivation-induced feeding at 1 and 2 h after injection (0–1 h: P = 0.0102, 0–2 h: P = 0.0027; Fig. 1). There were no significant main effects of treatment in the 1-
to 2-, 2- to 4-, and 4- to 24-h intervals or on cumulative 4- or 24-h food intake (data not shown).

Experiment 2A: The Effect of PVN UCN or CRH Administration on Nocturnal Feeding

There was a main effect of treatment on nocturnal feeding at 1, 2, and 4 h after injection (0–1 h: \(F_{2,26} = 6.075, P = 0.0068\); 0–2 h: \(F_{2,26} = 3.360, P = 0.0504\); 0–4 h: \(F_{2,26} = 10.470, P = 0.0005\); Fig. 2A). At 16 and 40 h after injection, there were also main effects of treatment on feeding (0–16 h: \(F_{2,26} = 4.221, P = 0.0259\); 0–40 h: \(F_{2,26} = 4.221, P = 0.0147\), data not shown). There were no treatment effects within the 16- to 40-h or the 40- to 64-h time intervals (data not shown), and thus intake was not monitored further.

Post hoc analysis of the 0- to 1-, 0- to 2-, and 0- to 4-h data indicate that UCN and CRH were approximately equally effective in decreasing feeding. However, in the 0- to 16-h interval, post hoc analysis indicated that CRH did not significantly decrease feeding (\(P = 0.2948\), data not shown), whereas the effect of UCN on feeding was significant (\(P = 0.0098\), data not shown).

Experiment 2B: Dose Effect of UCN Administered in the PVN on Nocturnal Feeding

There was a main effect of treatment on nocturnal feeding at 1 h after injection (\(F_{4,28} = 3.789, P = 0.0139\); Fig. 2B). Post hoc analysis of the data indicated that UCN at 10, 30, and 100 pmol significantly decreased nocturnal feeding (\(P = 0.0045\), \(P = 0.0156\), and \(P = 0.0031\), respectively; Fig. 2B). These data were converted to percent inhibition, using the aCSF mean food intake value as the control level of intake. The UCN doses were then plotted as a function of percent inhibition, and an \(IC_{50}\) dose was generated. For nocturnal feeding, the \(IC_{50}\) was 49 pmol UCN.

Experiment 3A: The Effect of PVN UCN or CRH Administration on NPY-Induced Feeding

There was a main effect of treatment on food intake at 1, 2, and 4 h after injection (0–1 h: \(F_{9,159} = 4.603, P = 0.0001\); 0–2 h: \(F_{9,159} = 3.183, P = 0.0016\); 0–4 h: \(F_{9,159} = 4.936, P = 0.0001\); Fig. 3A). Post hoc analysis indicates that at 1 h, all doses of UCN significantly decreased NPY-induced feeding (\(P < 0.05\); Fig. 3A), whereas only the 10 and 100 pmol doses of CRH resulted in a significant reduction in NPY-induced feeding (\(P < 0.05\); Fig. 3A). At 2 h, the results were somewhat similar, in that all doses of UCN significantly decreased NPY-induced feeding (\(P < 0.05\), data not shown). However, at this time, the effect of CRH on NPY-induced feeding was no longer evident. None of the doses of CRH significantly decreased NPY-induced feeding at 2 h (data not shown). At 4 h, the results were similar to those at 2 h: all of the doses of UCN signif-

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Figure 1. The effect of paraventricular nucleus (PVN) urocortin (UCN) administration on deprivation-induced feeding at 1 and 2 h after injection. Food intake is cumulative. *\(P < 0.05\) compared with control [artificial cerebrospinal fluid (aCSF)] group. \(n = 12\) per group.

Figure 2A: the effect of PVN UCN administration on nocturnal feeding at 1, 2, and 4 h after injection. Food intake is cumulative. *\(P < 0.05\) compared with control (aCSF) group. \(n = 14\) per group. B: dose effect of PVN UCN administration on nocturnal feeding at 1 h after injection. Food intake is cumulative. *\(P < 0.05\) compared with control (aCSF) group. \(n = 8\) per group.
Significantly decreased NPY-induced feeding \((P < 0.05; \text{Fig. 3A})\), whereas CRH was ineffective (Fig. 3A).

**Experiment 3B: The Effect of PVN UCN Administration on PVN NPY-Induced Feeding**

There was a main effect of treatment on food intake at 1, 2, 4, and 24 h after injection \((0–1 \text{ h}: F_{6,48} = 3.708, P = 0.0041, \text{Fig. 3B}; 0–2 \text{ h}: F_{6,48} = 4.566, \text{data not shown}, P = 0.001; 0–4 \text{ h}: F_{6,48} = 4.459, P = 0.0012, \text{respectively}, \text{Fig. 3B}; 0–24 \text{ h}: F_{6,48} = 3.891, P = 0.003, \text{data not shown})\). Post hoc analysis indicates that at 1 h, all doses of UCN significantly decreased NPY-induced feeding \((P < 0.05, \text{Fig. 3B})\). At 4 h, the 1 pmol dose no longer significantly decreased NPY-induced feeding, whereas 3, 10, 30, and 100 pmol UCN significantly decreased NPY-induced feeding \((P < 0.05; \text{Fig. 3B})\). There were no main effects of treatment on food intake in the 1- to 2- or 2- to 4-h intervals.

The 0- to 1-h data were converted to percent inhibition using the mean food intake for the NPY-treated rats as the control level of intake. The UCN doses were then plotted as a function of percent inhibition, and an IC\(_{50}\) dose was generated. For NPY-induced feeding, the IC\(_{50}\) was 26 pmol UCN.

**Experiment 4: The Effect of PVN UCN Administration on Preference for Saccharin Solution**

There was a main effect of treatment on the percent of fluid intake attributed to saccharin \((F_{3,22} = 4.475, P = 0.0154; \text{Fig. 4})\). Post hoc analysis indicates that intake of saccharin solution in the animals receiving 100 pmol UCN was significantly lower than intake of the animals receiving 0, 1, and 30 pmol UCN \((P < 0.05; \text{Fig. 4})\). Saccharin intake in animals receiving 1 and 30 pmol UCN was not different from that of control animals (Fig. 4).

**Experiment 5: The Effect of PVN UCN on c-Fos-ir in Several Forebrain Regions**

There were no main effects of UCN treatment on c-Fos-ir in any region measured (Table 1). These regions included the ACo, the Arc, the BNST, the CeA, the DMH, the LH, the LSi, the PVN, the SUMM, and the VMH.

**Experiment 6: The Effect of PVN UCN on NPY-Induced c-Fos-ir in Several Forebrain Regions**

There were main effects of treatment on c-Fos-ir in the Arc \((F_{2,23} = 4.214, P = 0.0276; \text{Fig. 5A})\) and the
PVN ($F_{2,19} = 4.477, P = 0.025$; Fig. 5B). However, as shown in Fig. 5, the effects observed were due to NPY treatment, and UCN had no affect on NPY-induced c-Fos-ir in these regions (Fig. 5, A and B). Post hoc analysis indicates that in the Arc, c-Fos-ir in the NPY-treated rats and the UCN + NPY-treated rats was significantly greater than the aCSF-treated animals ($P = 0.0151$ and $P = 0.0324$, respectively; Fig. 5A). There is a similar pattern in the PVN. NPY treatment resulted in a significant induction of c-Fos-ir ($P = 0.0033$, Fig. 5B), and UCN did not alter this induction, because c-Fos-ir levels in this group were also significantly above control levels ($P = 0.0284$, Fig. 5B). There were no main effects of treatment on c-Fos-ir in the CeA, the DMH, the NAccSh, or the VMH (Table 2).

**DISCUSSION**

UCN administration in the PVN was tested for effects on feeding in three separate paradigms: deprivation-induced, NPY-induced, and nocturnal feeding. The ability of PVN UCN to produce a CTA was also tested. UCN in the PVN significantly reduced feeding induced by NPY administered in the PVN at doses as low as 1 pmol UCN (Fig. 3B). Nocturnal feeding was also affected: 10 pmol UCN significantly decreased normal nighttime feeding (Fig. 2B). Compared with inhibition of nocturnal feeding, UCN had an approximately twofold greater efficacy in decreasing NPY-induced feeding: the IC$_{50}$ for UCN inhibition of NPY-induced feeding was 26 pmol, whereas the IC$_{50}$ for UCN inhibition of nocturnal feeding was 49 pmol.

**Table 1. Summary of results from experiment 5: the effect of PVN UCN on c-Fos-ir in several forebrain regions**

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Mean Number c-Fos-ir Cells</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCSF</td>
<td>UCN</td>
<td></td>
</tr>
<tr>
<td>ACo</td>
<td>124 ± 64</td>
<td>0.7693</td>
</tr>
<tr>
<td>Arc</td>
<td>130 ± 37</td>
<td>0.0948</td>
</tr>
<tr>
<td>BNST</td>
<td>109 ± 45</td>
<td>0.6605</td>
</tr>
<tr>
<td>CeA</td>
<td>23 ± 9</td>
<td>0.4240</td>
</tr>
<tr>
<td>DMH</td>
<td>48 ± 15</td>
<td>0.5194</td>
</tr>
<tr>
<td>LH</td>
<td>71 ± 23</td>
<td>0.3919</td>
</tr>
<tr>
<td>LSI</td>
<td>109 ± 26</td>
<td>0.5315</td>
</tr>
<tr>
<td>PVN</td>
<td>309 ± 65</td>
<td>0.5161</td>
</tr>
<tr>
<td>SUMM</td>
<td>116 ± 48</td>
<td>0.2481</td>
</tr>
<tr>
<td>VMH</td>
<td>53 ± 24</td>
<td>0.6648</td>
</tr>
</tbody>
</table>

Values are means ± SE. ACo, cortical nucleus of the amygdala; Arc, hypothalamic arcuate nucleus; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; DMH, dorsomedial hypothalamus; LSI, intermediate portion of the lateral septal nucleus; PVN, hypothalamic paraventricular nucleus; SUMM, medial supramammillary nucleus; VMH, ventromedial hypothalamus; aCSF, artificial cerebrospinal fluid; UCN, urocortin; ir, immunoreactivity.

**Table 2. Summary of results from experiment 6: the effect of PVN UCN on NPY-induced c-Fos-ir in several forebrain regions**

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Mean Number c-Fos-ir Cells</th>
<th>P Value (Main Effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arc</td>
<td>aCSF + aCSF</td>
<td>aCSF + NPY</td>
</tr>
<tr>
<td>CeA</td>
<td>69 ± 19</td>
<td>154 ± 26</td>
</tr>
<tr>
<td>DMH</td>
<td>32 ± 8</td>
<td>71 ± 16</td>
</tr>
<tr>
<td>NAccSh</td>
<td>118 ± 34</td>
<td>182 ± 37</td>
</tr>
<tr>
<td>PVN</td>
<td>330 ± 96</td>
<td>1055 ± 159</td>
</tr>
<tr>
<td>VMH</td>
<td>123 ± 31</td>
<td>176 ± 27</td>
</tr>
</tbody>
</table>

Values are means ± SE. $P$ value represents significance of difference between neuropeptide Y (NPY) and UCN+NPY groups. NAccSh, shell of the nucleus accumbens.
These doses are within range of the ED$_{50}$ of 0.27 $\mu$g (~60 pmol) previously reported for feeding inhibition by intracerebroventricularly injected UCN (12). Feeding inhibition by intracerebroventricularly injected UCN may represent actions within the PVN, inasmuch as the PVN is relatively accessible to intracerebroventricularly injected compounds. However, at the same dose, efficacy of compounds injected into specific sites is usually much greater than efficacy observed after ventricular injection. It is likely that there are other, as of yet unidentified sites responsive to UCN feeding inhibition, which is possible because there are other brain sites containing UCN circuitry.

In the nocturnal and NPY feeding studies, UCN feeding inhibition occurred at doses much lower than those required to produce a CTA (Fig. 4). In contrast, 100 pmol UCN was required to significantly decrease deprivation-induced feeding (Fig. 1). This dose resulted in a CTA in the two bottle test (Fig. 4), which leaves open the possibility that the decreased feeding observed in these animals was due to an aversive event. Another explanation for this high dose requirement may be related to the strength of the stimulus. In the first hour of feeding induced by PVN NPY and nocturnal feeding, the level of food intake was approximately one-third that observed in the first hour after the food-deprived animals were given access to food. Because the first hour is the time in which UCN is most effective in other feeding paradigms and the feeding stimulus in this time period in previously food-deprived animals is very strong, UCN may be unable to adequately influence this powerful stimulus. Nonetheless, the ability of UCN to decrease nocturnal and NPY-induced feeding at doses much lower than that required to produce a CTA supports the hypothesis that UCN may be a satiety factor.

UCN is closely related to CRH and binds to the same receptors, albeit more avidly than CRH. Thus the UCN-NPY interaction indicated here might have been predicted based on previous studies suggesting PVN CRH inhibition of NPY feeding pathways (4, 6, 7). The current UCN and NPY coinjection data suggest that UCN inhibits NPY downstream signaling pathways in the PVN to influence feeding (Fig. 3). Our data also demonstrate that although UCN is approximately equipotent to CRH in decreasing spontaneous feeding (Fig. 2A), it is more potent and longer lasting than CRH in depressing NPY-induced feeding (Fig. 3A). However, in the current PVN studies, there was not a 25-fold difference in efficacy between UCN and CRH as was observed in feeding studies after ventricular UCN and CRH injections (12).

Ventricular UCN administration results in elevated c-Fos-ir in several brain regions, including the LSI, dorsal raphe nuclei, and nucleus of the solitary tract (13), and may indicate that the anorectic response to UCN is due to engagement of feeding-inhibitory pathways within these brain sites. However, in the current study, UCN alone in the PVN did not influence c-Fos-ir in the LSI, nor in several other forebrain regions (Table 1). Because forebrain sites were not influenced (Table 1), further analysis of hindbrain sites was not performed. The discrepancy between the current c-Fos-ir data after PVN-injected UCN and that of ICV-injected UCN likely relates to the neural origin of UCN stimulus. After NPY administration in the PVN, there was significant elevation of c-Fos-ir in sites associated with PVN-injected NPY (Fig. 5, A and B; Table 2) (9). However, UCN in the PVN did not alter this response (Fig. 5, A and B; Table 2). Thus, although the current feeding studies indicate that UCN in the PVN blocks feeding induced by NPY administered into the PVN (Figs. 1–3), UCN does not block elevations in c-Fos-ir induced by NPY administered into the PVN. An important difference between the feeding studies and the c-Fos-ir studies reported here is that food was allowed postinjection in the feeding studies but was not allowed postinjection in the c-Fos-ir studies. It is possible that UCN influence on PVN NPY-stimulated c-Fos-ir in specific brain sites occurs only when pathways involved in food ingestion are activated. Another explanation for these data may be that UCN in the PVN acts on different cells than NPY, with subsequent interaction between these cell populations.

In conclusion, we have identified the PVN as responsive to UCN anorectic action. UCN in the PVN decreases feeding in several paradigms and appears to be particularly effective in decreasing NPY-stimulated feeding. These results support the hypothesis that UCN is an endogenous anorectic peptide and are consistent with the major role of the PVN in the regulation of energy balance. However, because both UCN and CRH are present in the PVN and both peptides bind to CRH receptors, the relative contribution of each of these peptides in the PVN to signaling satiation is unknown. Studies with specific CRH receptor subtype antagonists, gene targeted knockouts, or other peptide and/or receptor deactivation methods will help to unravel the functional domains of UCN and CRH. Finally, because the anorectic response to ventricular UCN is more potent than that after ventricular CRH administration, it is likely that there are sites other than the PVN that are sensitive to UCN anorectic properties.

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